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# The Effect of Silver Coating Thickness on Cytotoxicity Nickel-Chromium Alloys as Fixed Denture Materials

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NiCr alloy, silver coating, viability, fibroblast.

**ABSTRACT**

Nickel-chromium (NiCr) alloy is commonly used for fixed partial denture material. However, these alloys can undergo corrosion which can affect biocompatibility. One of the coatings that can be used is silver. Silver has good biocompatibility, antifungal and minimal allergic potential. This study aimed to examine the effect of the silver coating thickness on nickel-chromium alloys as adhesives on cytotoxicity. The sample consisted of 28 NiCr alloys with disc shape (diameter 5mm; width 2mm), divided into four groups (n=6), namely group I control (NiCr alloy without coating), group II NiCr alloy metal with a silver coating thickness of 1µm, group III is a NiCr alloy with a silver coating of 5µm thickness, and group IV is a NiCr alloy with a silver coating of 10µm thickness. Observing surface morphology and thickness of a silver coating by scanning electron microscope (SEM) used four samples. Cell viability was obtained using the MTT assay method and examined with a microplate reader. The results were analyzed using one-way ANOVA, followed by a post hoc LSD test. The results showed the highest percentage of cell viability in the 5µm thickness silver coating group with a value of 78.93%. The one-way ANOVA and post hoc LSD test showed significant differences between groups ($p<0.05$). This research concludes that 5µm is the optimal thickness of the silver coating on nickel-chromium alloy as a fixed partial denture material to reduce cytotoxicity.

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## 1. INTRODUCTION

Loss of teeth will affect the patient's appearance, so it becomes the main reason for denture treatment [1]. Various treatment options can be carried out to replace missing teeth. Removable or fixed dentures are the two main options for restoring function and esthetics [2]. Fixed dentures are restorations permanently installed in the remaining teeth to replace one or more missing teeth [3]. Restoration with fixed dentures can satisfy patients and dentists because it is more comfortable, restores occlusion function, and improves patient esthetics [4]. Materials often used for fixed denture is porcelain fused to metal. Restoration with this material is still a good option in some cases of fixed prosthodontics [5].

Fixed partial denture materials with porcelain fused to metal materials generally use nickel-chromium (NiCr) alloys as retainers due to resin cements high shear bond strength. In addition, the alloy has sufficient hardness and good physical and mechanical properties [6], [7]. Another advantage of this alloy is its much higher elasticity modulus than precious metals. Therefore, long fixed dentures made of nickel-chromium (NiCr) alloys will be subjected to much lower stresses than similar dentures made of precious metal alloys, with a lower probability of fracture of the porcelain components [8].

Nickel-chromium alloys have a disadvantage, and they can corrode and potentially cause allergic or other tissue reactions [9]. Saliva and crevicular fluids can cause corrosion of nickel-chromium alloys. Corrosion is closely related to the biocompatibility of metal alloys. Contact between saliva and crevicular fluid with dental metal materials will result in corrosive metal ions resulting in precipitation of metal ions and their derivatives, which will cause harmful effects on body tissues [10].

The biocompatibility of metal alloys used as fixed partial denture materials must be considered because these materials are always in contact with oral tissues [11]. Generally, dental materials used to replace missing or missing tooth tissue must be chemically stable and inert to the oral cavity. When materials in dentistry experience component release, these components will cause toxicity, making it possible for local and systemic tissue reactions [4].

Modifications by coating metal materials can be done to protect and increase corrosion resistance. For example, coated nickel-chromium alloy with titanium metal (Ti) can limit the release of metal ions [12]. A coating nickel-chromium alloy with titanium metal (Ti) reduces the cytotoxicity effect on fibroblast cells [13]. In addition, zirconia (Zr) coatings can reduce the corrosion potential of nickel-chromium alloys [14].

Silver (Ag) can be an alternative to other metal coatings. Silver (Ag) is a precious metal that is biocompatible and relatively safe for the human body [15]. Silver is a metal that has less allergic potential than metals. Others have been used as coating materials, such as titanium and chromium. The potential for silver ion release is relatively low and non-toxic [16], [17]. Silver-containing materials are chemically stable at high temperatures, and releasing silver ions takes a very long time [18].

The electroplating method will produce a dense and uniform silver layer, and no porosity is found on the silver surface. Silver coatings have good biocompatibility in long-term use [19]. Silver coatings with 10μm thickness are effective against bacteria [20]. Silver coatings on stainless steel with a thickness of 1μm can also prevent the release of nickel and chromium ions [21]. Cobalt chromium alloys with 5μm and 10μm thickness can also control the release of metal ions [22].

The potential of a material as a cause of damage can be determined through a cytotoxicity test by looking at the percentage of cell viability [7]. In vitro tests are needed to measure and see cell viability [23]. In vitro examination is a type of examination carried out in test tubes and plates. Cell culture or outside the body of living things [18].

Cell viability tests in dentistry mostly use fibroblast cells [24]. Fibroblast cells are cells found in connective tissue that play a role in developing, maintaining, and repairing connective tissue [25]. In addition, fibroblast cells are easy to culture because they can grow. And high adhesion and rapid regeneration, so they are used to test cytotoxicity [26].

## 2. Material and Method

***2.1 Preparation of sample***

Samples were made using the casting technique of nickel-chromium alloy 4all (Ivoclar Vivadent, Germany) with a disc shape of 5mm diameter and 2mm thickness in as many as 24 pieces. Then the sample was polished with silicon carbide paper then cleaned for 15 minutes using alcohol to clean the specimen from contamination.

**Figure 1.** Sample of nickel-chromium alloy with a diameter of 5mm and a thickness of 2mm

***2.2 Electroplating of silver***

An electrochemical reaction carries out the silver galvanic deposition technique. The electrolyte source of silver ions used in this process consists of silver nitrate, sodium phosphate and ammonium phosphate. The silver ions are positively electrified (anode), which is transferred to the electrolyte solution and flowed to the surface of the nickel-chromium metal as the cathode. The current used in the electroplating process is 0.25 Ampere.21 The length of the electroplating process for a thickness of 1µm for 1 minute, 5µm for 5 minutes, and 10µm for 10 minutes.

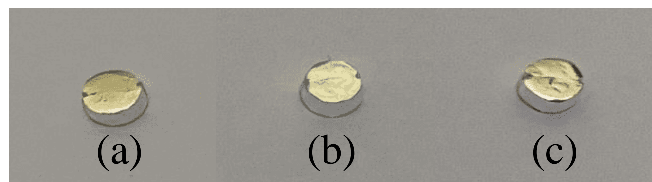


**Figure 2.** Nickel-cromium alloy coated with silver with a thickness of (a) 1µm; (b) 5 µm; (c) 10µm

***2.3 Cell viability test***

Research subjects were put into each microplate well containing 100µl of artificial saliva with the composition of NaCl 400mg/l, KCL 400mg/l, CaCl2.2H2O 795 mg/l, NaH2PO4.H2O 690mg/l, Na2S.9H2O 5mg/l urea (CH4N2O) 1000mg/l with a pH of 7.1. Each well was then marked according to the treatment group. Then the microplate was stored in an incubator for 24 hours at $37^0$C [27].

Saliva in the wells was aspirated with a micropipette and then filled with fibroblast cells with a density of 2 x 104 cells/100µl. Incubate for 24 hours in an incubator. The culture media was discarded, and each well was given 100µl of MTT solution and incubated for 4 hours at $37^0$C. 100µl of DMSO was added to each well, and the absorbance was read with a microplate reader with a wavelength of 570nm.

***2.4 Surface analysis***

Surface observations using a scanning electron microscope (SEM) to see the surface of the silver layer formed on the nickel-chromium alloy metal.

***2.5 The thickness of silver coating analysis***

Observation of the thickness of the silver coating was carried out using a scanning electron microscope (SEM) so that an assessment of the thickness of the silver layer on nickel-chromium alloys could be carried out.

***2.6 Statistical analysis***

Data were analyzed by one-way ANOVA with a significance level of 95%, followed by Fisher's least significant difference (post hoc LSD) test. In addition, the morphology of the silver layer and the thickness of the silver layer were analyzed qualitatively.

## 3. Result

### *3.1 Cell viability test*

Absorbance examination using a microplate reader with a wavelength of 570nm. The mean values of fibroblast cell viability after exposure to nickel-chromium alloy metal coated with silver showed in table 1.

**Table 1.** The results of the average viability of fibroblast cells (%) after exposure to nickel-chromium alloy metal with silver plating with a thickness of 1μm, 5μm, and 10μm

| Group | Mean ± SD (%) |
|---|---|
| Control | 70,47 ± 0,29 |
| 1μm | 71,35 ± 0,49 |
| 5μm | 78,93 ± 0,45 |
| 10μm | 76,14 ± 0,83 |

### *3.2 Surface analysis*

Surface observations were carried out using a scanning electron microscope (SEM). The surface observed with a magnification of 5000x is shown in Figure 3. Observations show that the silver layer with a thickness of 1μm shows an image of the surface of the silver layer that is not dense and it is still porous between silver particles. The surface depiction of 5μm thick silver coating shows the surface of the silver layer is denser and more evenly distributed. The thickness of the silver coating was 10μm, and the surface image with different sizes of silver particles

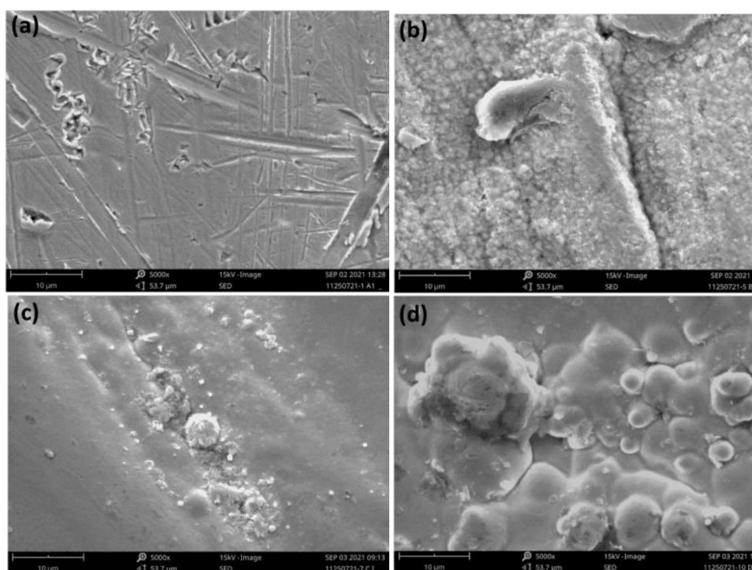


**Figure 3.** Scanning electron microscope (SEM) image of the sample surface. (a) Uncoated nickel-chromium (NiCr) alloy metal; (b) Nickel chromium alloy with a silver plating of 1μm thickness; (c) Nickel chromium alloy metal with a silver coating of 5μm thickness; (d) Nickel chromium alloy metal with 10μm thickness silver coating.

### *3.3 The thickness of silver coating analysis*

The thickness of the silver coating was observed using a scanning electron microscope (SEM) with a magnification of 750x (Figure 4). The picture shows that the silver layer on nickel-chromium alloys with different thicknesses can bond with nickel-chromium alloys

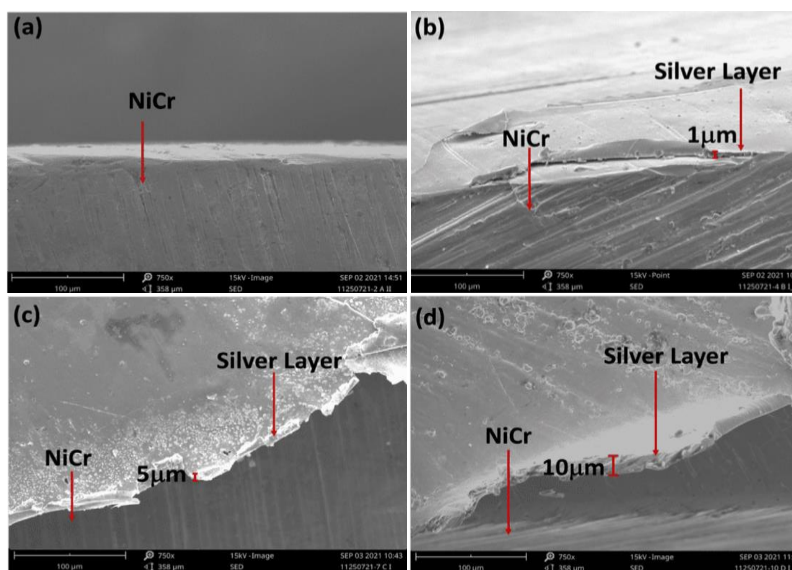


**Figure 4.** Cross section of nickel-chromium alloy metal. (a) Uncoated and silver plated with thickness; (b) 1μm; (c) 5μm; (d) 10μm

### *3.4 Statistical analysis*

The Shapiro-Wilk normality test and the Levene homogeneity test obtained data that were normally distributed and homogeneous ($p>0.05$). The results of the one-way ANOVA test obtained p value $<0.05$, showed a significant difference in the viability of fibroblast cells between the control group (NiCr), nickel-chromium alloy metal with 1μm, 5μm, and 10μm silver coatings. In addition, the post hoc LSD test results in table 2 show a significant difference in the viability value of fibroblast cells in all groups ($p<0.05$).

**Table 2.** Results of Fisher's least significant difference (post hoc LSD) fibroblast cell viability

| Group | Mean difference | | | |
|---|---|---|---|---|
| | Control | 1μm | 5μm | 10μm |
| Control | - | -0,8783* | -8,4533* | -5,6700* |
| 1μm | - | - | -7,5750* | -4,7916* |
| 5 μm | - | - | - | 2,7833* |
| 10 μm | - | - | - | - |

## 4. Discussion

The potential for dental materials to cause damage when applied to cells can be determined through a cytotoxicity test by looking at the percentage of cell viability. The table of mean viability shows that all groups in this study are included in the non-toxic category. These results are in accordance with [28], and more than 70% cell viability is categorized as non-toxic. [29] also stated that the higher the viability value, the higher the number of living fibroblasts. The percentage of live fibroblasts indicates the level of toxicity of a material. The higher the percentage of fibroblast cell viability, the lower the cytotoxicity of a material.

The one-way ANOVA test results showed a significant difference in the mean viability in all groups ($p<0.05$). The results of this study indicate that the thickness of the silver coating affects the cytotoxicity of nickel-

chromium alloys. The silver coating can reduce cytotoxicity, possibly due to the presence of a silver layer distributed on the surface of nickel-chromium alloys. The silver coating on the surface of the alloy metal acts as a protective layer to prevent the release of potentially toxic alloying elements [7], [30].

The silver coating can reduce the cytotoxicity of nickel-chromium alloys, possibly because silver is a material that has good biocompatibility. This follows previous studies stating that silver is a precious metal that is biocompatible and relatively safe for the body [15]. [13] also noted that nickel-chromium alloy coating could reduce its cytotoxicity to fibroblast cells. In addition, silver also has good biocompatibility. [31] also stated that silver coating has good biocompatibility against fibroblast cells. This is also supported by [32], who stated that silver is safe for mammal cells, and calcium phosphate coatings containing silver will benefit orthopaedic implants.

The post hoc LSD showed the lowest viability value of the uncoated nickel-chromium alloy, with a viability value of 70.47%. This may be caused by fibroblast cells in direct contact with nickel-chromium alloys and the possibility of releasing elements from the alloy metal that can cause fibroblast cell death. In addition, elements that are released from these alloys will interfere with the ability of cell metabolism. [33] also stated that elements released from nickel-chromium alloys would accumulate in nearby tissues and cause inflammation. These results are supported by previous studies, which stated that nickel-chromium alloys could potentially have high cytotoxicity, resulting in cell damage [34].

The post hoc LSD test results showed that the 10μm thickness of the silver coating had a lower viability value than the 5μm thickness, probably due to the number and size of silver particles formed on the silver layer. This is supported by the scanning electron microscope (SEM) image, which shows that the sample's surface forms a silver layer with various particle sizes. Electroplating coating techniques will produce layers with multiple particle sizes and fill in the gaps between the particles present [35]. The number of particles, particle size, and state of aggregation of silver particles is an essential factors in the toxicity of silver particles [36]. In addition, silver particle size is a significant factor in mediating various biological effects, such as oxidative stress, DNA damage, cellular uptake, mitochondrial dysfunction, and permeability of biological barriers [37].

The average size of silver particles formed by electroplating is 35–55nm and has a globular structure [38], [39]. The standard size of silver particles that do not cause cytotoxicity is particles with a diameter of 1–100nm [36]. Smaller silver particles have more cytotoxicity. Higher than the larger silver particles. Small particles have a higher ability to enter the cell nucleus through the nuclear membrane so that they can affect cell bioactivity [40]. The 10μm thick silver coating group in this study may have a larger number of small particle sizes than the 5μm silver coating thickness. This study has limitations, not measuring the number and size of particles in the silver layer.

The 10μm thickness silver layer has a lower viability value than the 5μm thickness, possibly because the number of silver particles in the 10μm layer thickness is higher and has toxic potential. Intracellular silver particles induce oxidative stress, cell membrane damage, inflammatory processes, DNA damage, chromosomal aberrations, and apoptosis [41]. Exposure to silver particles can cause changes in cell shape, decrease cell viability, and increase lactate dehydrogenase (LDH) production, which ultimately results in apoptosis and necrosis. The toxic potential of silver particles is relatively lower than that of nickel and chromium particles. The results of the previous study by [17] stated that the potential for releasing silver particles is relatively low. [18] also stated that materials containing silver are chemically more stable at high temperatures and the process of releasing silver particles is longer than metal alloys.

The results of the post hoc LSD test of nickel-chromium alloy metal with a silver coating of 1μm thickness have a lower viability value than those with a thickness of 5μm and 10μm, possibly due to the more porous formed on the surface of the silver layer with a thickness of 1μm so that the potential for the element to be released from the nickel-chromium alloy is higher. This is supported by observations of silver thickness using a scanning electron microscope (SEM) which shows a thickness of 1μm containing a porous part between silver particles. [42] stated that in a 1μm thick coating, only a few silver particles were deposited on the surface of the alloy metal so that there were still porous parts. [43] reported that the film has a porous layer structure, so it cannot act as a full shielding element from an alloying metal.

The porosity formed on the silver layer with a thickness of 1μm may be due to the varying size of silver particles formed by electroplating. The silver layer formed on the substrate by electroplating will produce varying particle sizes [20]. The large particles formed may be the reason for porosity formation in the silver layer. In addition, the porous part of the surface of the silver layer may cause the elements to be released from the nickel-chromium alloy. Elements released from nickel-chromium alloys can initiate reactive oxygen species (ROS) formation and damage to mitochondrial membranes, which act as important factors in the early stages of cell death [44].

LSD post hoc test results also showed that nickel-chromium alloy metal with 5μm silver coating showed the lowest cytotoxicity results. This is probably because the silver layers formed at a thickness of 5μm are solid, so they can be an effective protective layer to prevent the release of potentially toxic metal elements. These results are based on previous studies, which stated that silver coating on cobalt-chromium alloys with a thickness of 5μm and 10μm prevented the release of metal ions [22]. [42] stated that by increasing the electroplating process time, Gradually, the porous part of the silver layer will be filled with silver particles. As the thickness of the silver coating on the alloy increases, the porosity of the silver coating will decrease [45]. [46] also stated that increasing the number of layers can be used for surface protection of a material which will affect the release of ions in a material. Based on the results of post hoc LSD, it was found that 5μm thickness was the optimal thickness for reducing cytotoxicity.

## 5. Conclusion

The thickness of the silver coating on nickel-chromium alloy as a fixed partial denture material decreased cytotoxicity with an optimal thickness of 5μm.

## 6. Suggestion

a. Further research can be carried out with a scanning electron microscope (SEM) to determine the particle size of the silver coating.

b. Further research can be carried out to determine the number of silver ions released on silver coating using the electroplating method.

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